Environmental Risk Factors for Osteoporosis

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Environmental risk factors for osteoporosis were reviewed at a conference held at the National Institute for Environmental Health Sciences 8–9 November 1993. The conference was co-sponsored by the National Institute of Arthritis and Musculoskeletal and Skin Disease and the NIH Office of Research in Women's Health. The objective of the conference was to review what is known about risk factors for osteoporosis and to identify gaps in the present state of knowledge that might be addressed by future research. The conference was divided into two broad themes. The first session focused on current knowledge regarding etiology, risk factors, and approaches to clinical and laboratory diagnosis. This was followed by three sessions in which various environmental pollutants were discussed. Topics selected for review included environmental agents that interfere with bone and calcium metabolism, such as the toxic metals lead, cadmium, aluminum, and fluoride, natural and antiestrogens, calcium, and vitamin D.

Bone Metabolism and Osteoporosis

The pathogenesis of fractures in osteoporosis is complex; low bone mass is the critical factor. Microarchitectural abnormalities (loss of the interconnectivity of trabeculae) also play a role. A change of 1 SD below the mean for people without osteoporosis increases the risk of bone fracture 1.5–2.5 times. Epidemiology studies have identified estrogen deficiency and aging as major risk factors for women, but reasons for bone loss in males are not understood. Although 70-80% of peak bone mass appears to be genetically determined based on studies of twins, there is no apparent genetic component to the rate of bone loss. There is need for therapies that will enhance bone formation. Another question is whether there should be universal estrogen therapy for post-menopausal women and whether we can influence lifestyle practices such as dietary calcium intake and exercise in childhood and adolescence, which are the critical bone-forming years. Finally, the potential effectiveness of bisphosphonates and fluorides in preventing osteopenia and increasing bone mass needs to be determined.

The use of absorptiometry and other techniques for measuring bone density and assessing fracture risk was reviewed. Fracture occurrence is related to bone strength and the load applied to the bone. This in turn is related to gait speed, soft tissue padding, and direction and height of fall. Bone mass (density) is probably the major determinant of bone strength and can be measured by a variety of noninvasive techniques including ultrasound. However there are problems of reproducibility (precision), site specificity, and fracture predictability. Quality of bone is also an important determinant of fracture risk, but it cannot be assessed by noninvasive methods.

Serum and urinary markers of bone mineral metabolism can be divided into those that reflect bone formation and those that are the product of bone resorption. The markers, especially osteocalcin, also have to be understood in the context of bone remodeling where formation is coupled to resorption and mineral homeostasis. Markers of bone formation that are most commonly used in research and clinical practice are alkaline phosphatase, osteocalcin, and procollagen type 1 peptides. Alkaline phosphatase suffers from nonspecificity but is still the best test of osteoblast function, as in Paget's disease of bone. There are now a number of immunoassays for osteocalcin, the standard for measuring osteoblastic activity. Osteocalcin generally correlates with alkaline phosphatase and bone histomorphometry. Problems are that the function of osteocalcin is not entirely understood, and there is no international standard regarding specificity to objectively compare the various immune assays that are available. Blacks have higher levels of osteocalcin than whites. There are also problems with storage of osteocalcin and changes during circadian rhythms and menstrual cycles. There is also a contribution of osteocalcin from platelets that is not related to bone. Tests for type 1 procollagen peptides are new and not fully evaluated.

Serum tartrate resistant acid phosphatase is a marker for bone resorption but is tedious to measure because changes are small. Serum telopeptides of type 1 collagen are also available but are not fully evaluated. There are a number of urinary markers of bone resorption. The most reliable are measurements of D-pyridinoline and total pyridinoline cross-links. These correlate with bone densitometry and histomorphometry. Measurements may be influenced by liver and renal disease. A serum assay is being developed that will allow these cross-link measurements to be included in a battery of markers including 1,25-vitamin D, parathyroid hormone, and osteocalcin. Other tests of bone resorption are urinary hydroxyproline (influence by diet) and urine calcium creatinine ratios. Although any one of these markers is not sufficient as a predictor or diagnostic tool of bone loss, a panel of tests may define people at risk for excess bone loss and prompt intervention strategies.

Among experimental models for studying bone disease, the rat may be the preferred model for a number of reasons. To be suitable, an animal model should either develop low bone mass experimentally or lose bone similarly to the human situation for osteoporosis. The rat is the most widely used animal model because methods for analysis of rat skeletal tissue are fairly standard, results from experiments are consistent, and the rat skeleton accurately reflects disease states found in humans. Rats also provide a rapid time frame to assess results of studies. Environmental effectors can easily be applied to rats. Finally, the cost of conducting studies on rats are reasonable. Nevertheless, there are several questions and problems regarding the rat, such as lack of evidence of fractures from osteoporosis, lack of a Haversian system, and lack of suitable genetic markers, although these may be provided in time. One criticism is that rat bones grow continuously. Nevertheless, rat bone undergoes modeling and remodeling similar to humans.

The role of vitamin D as a factor in the etiology of osteoporosis was debated. Clinical trials with vitamin D have shown promise, especially in selected populations where vitamin D levels are deficient or marginal. In normal populations, optimal dose and risk of hypercalcemia need to be defined. A related benefit of vitamin D may be its immunoregulatory and cell-differentiating properties.

Fetal rat calvaria is a common model for studying bone formation and the

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development of the osteoblast phenotype. The action of steroid hormones, vitamin D, glucocorticoids, and estrogens on the osteoblast can be further clarified using biochemical and molecular approaches *in vitro*. These techniques have shown that certain hormones affect different genes depending on the stage of osteoblast development and maturation, secondary tissue organization, and rate of differentiation. The osteocalcin gene has been the focus of attention regarding regulation by steroid hormones, which may provide some insight as to therapy of osteoporosis.

Environmental Risk Factors

Lead

Lead is a potential risk factor for osteoporosis because of the central role the skeleton plays in lead toxicokinetics, and as well as being a target tissue for lead toxicity. The four presentations emphasized the diverse effects of lead on bone cell function, the toxicokinetics and toxicology of lead in bone, the state of the art for noninvasive measurements of lead in tibia and other bones, and the use of stable isotope techniques to determine the contribution of skeletal lead to blood lead.

Numerous *in vitro* and *in vivo* studies have established the osteoblast and osteoclast as targets of lead toxicity. Lead has complex effects on bone cell function. First, lead may indirectly alter bone cell differentiation and function by altering the plasma levels of calciotropic hormones, especially 1_{α},25-dihydroxyvitamin D_3 and parathyroid hormone. Second, lead may directly alter bone cell function by perturbing the ability of bone cells to respond to hormonal stimuli such as the synthesis of bone matrix proteins by perturbing calcium-mediated and other sensitive signal transduction pathways. Third, lead may directly interfere with hormone and cytokine signal transduction processes and thereby uncouple osteoblasts and osteoclasts from normal paracrine control. Fourth, lead may directly perturb bone cell function via biochemical inhibition of enzymes, resulting in altered cellular energetics. Thus, the cellular and molecular effects of lead on bone cells are, like the effects of lead on other biological targets, complex and multifaceted.

It has long been recognized that the skeleton contains most of the body burden of lead, and the skeleton is increasingly recognized as a target organ system for lead toxicity. The three most likely mechanisms by which lead might contribute to the development of osteoporosis include alteration of 1) peak bone mass, 2) the rate of bone resorption in older persons, and/or 3) alteration of the structural integrity of bone. There are limited experimental studies addressing each of these potential mechanisms. Although there is good support for retardation of endochondral bone growth by lead, the long-term consequences of this retardation on peak skeletal mass remain to be established. Unfortunately, the experimental and human studies are not sufficiently rigorous or detailed to either support or to eliminate a role for lead in the pathogenesis of osteoporosis.

In many circumstances, blood lead, which primarily reflects recent lead exposure, may be an inadequate indicator of lead cumulative exposure. Conceptually and experimentally, bone lead should provide a superior estimation of cumulative lead exposure. The noninvasive determination of lead in bone by X-ray fluorescence using photons from a radioisotope or X-ray generator to produce characteristic X-rays from lead in tibia, calcaneus, and other bones is a powerful predictor for quantifying past lead exposures and body burdens, primarily in individuals occupationally exposed to lead. Improved instrumentation in electronics and detectors is expected to provide sufficient sensitivity to be applicable to individuals and populations without excessive lead exposure. Studies in Baltimore suggest that a significant number of women may have bone lead levels >20 mg/g bone. Thus the use of bone lead measurements as a substitute/supplement for blood lead measurements as an index of lead exposure should prove useful in assessing the contribution of lead to diseases such as osteoporosis, renal disease, and heart disease.

Mobilization of lead from skeletal stores during pregnancy and lactation may constitute a potential threat to the fetus during critical stages of organ development. Stable isotope techniques are powerful for evaluating the contribution of skeletal lead stores to blood lead in humans, if there is a sufficiently large isotopic difference in the composition of skeletal lead and environmental lead. A pilot study has shown that migrants to Australia from Eastern Europe may be a satisfactory population for study, as the skeletal lead has the stable lead isotopic profile of Eastern Europe, while the Australian environmental lead has a much different stable isotope profile. Stable isotope analysis of the blood of these immigrants provides a reliable estimate of the contribution of skeletal lead to blood lead during pregnancy and lactation.

Calcium and Estrogen

Using stable isotope analysis of nonhuman primates, it was found that lead was mobilized from bone during pregnancy, producing internal lead exposure or dosing. Stable isotope analysis may be useful in determining which dietary or toxic or physiological processes are important in the mobilization process. An ongoing study will investigate the influence of different levels of dietary calcium on mobilization/retention of bone lead.

A positive calcium effect has been shown in every study in which calcium intake was controlled and in which subjects in the immediate post-menopausal period were excluded. The level of dietary calcium during the third decade of life was important in determining bone density and risk of osteoporosis and fractures later in life. Dietary sodium increases urinary calcium loss. In the United States there is a high sodium (salt) usage, which can be a negative risk factor for calcium metabolism and development of osteoporosis. Smoking and alcohol abuse also decrease bone mineral content. Estrogen is an important determinant of bone density, and calcium supplementation cannot repair other nutrient deficiencies, offset estrogen deprivation, or neutralize the untoward effects of unhealthy habits other than low consumption of food sources of calcium.

Regarding the regulation of estrogen receptor expression and responsiveness in osteoblasts, several steroid hormones affect bone in different ways. Estrogen is believed to act directly on estrogen receptors in bone cells. Levels of estrogen receptor can be modulated by retinoids influencing responsiveness to type I collagen and IGF-1 expression. Results of recent research suggest an interplay between different hormone signaling pathways, as occurs between protein kinase C and estrogen in bone cells.

In experimental models, transient exposure to estrogen during early developmental periods affects adult bone density by influencing osteoblast responsiveness to steroid hormones. Exposure to diethylstilbestrol and other environmental estrogens and estrogen agonists can induce changes in bone density and levels of estrogen during developmental periods and can dramatically influence cell programming, resulting in changes in the skeleton during adulthood. Studies in estrogen-receptor mutant animals indicate that lower bone density results from nonfunctional estrogen receptors.

Cadmium

The CADMIBEL study, conducted in Belgium, included a population of 2300 subjects, half of whom lived in areas with increased environmental exposure to cadmium due to their proximity to past and present zinc smelter operations. Cadmium in urine (Cd-U), used as an indicator of cadmium body burden, was shown 50–85% higher in residents of North Kempen, the most polluted area, than in residents of

Charleroi, the least polluted area. With respect to whether the cadmium exposures may have caused renal changes, five urinary markers of renal tubular dysfunction were significantly and positively associated with Cd-U, namely, retinol-binding protein (RBP), *N*-acetyl-glucoaminidase (NAG), β_2-microglobulin, total amino acids, and calcium. Further, it was estimated that the risk of calciuria and slight tubular effects was about 10% (that is, a relative risk of 2) when the daily urinary cadmium exceeded about 2 mg for calciuria, 3 mg for urinary NAG, RBP, and β_2-microglobulin, and about 4 mg for aminoaciduria. The low value for calciuria indicates that urinary calcium may be one of the earliest renal parameters to be affected by increasing cadmium body burden. By these criteria, results also indicated that about 10% of the general population of Belgium has a cadmium body burden sufficient to cause slight changes in calcium metabolism, with the percentage rising to 40% among 60- to 80-year-old women in North Kempen, the region of highest cadmium exposure.

In response to these results, the follow-up PHEECAD study is currently underway, involving about 1000 subjects from Hechtel-Eksel (rural control area) and North Kempen (rural cadmium exposure area). One question that PHEECAD addresses is whether the increased calciuria and serum alkaline phosphatase activity seen in the CADMIBEL study are associated with an increased risk of abnormal bone metabolism, as measured by forearm densitometry and serum and urine markers of bone remodeling.

Finally, in a group of 37 chronically exposed cadmium workers and 43 matched controls, with workers having Cd-U values five times higher than controls (5.4 versus 0.7 µg/g creatinine), urinary eicoanoids and sodium, but not calcium, were weakly but positively correlated with Cd-U. Because spot urine samples were collected, these results do not conclusively support or contradict results of the CADMIBEL study, in which 24-hr urine samples were taken.

The first of three studies on osteopenia and Itai-Itai disease in Japan involved 234 women and men with cadmium-induced renal dysfunction, including 28 women with Itai-Itai disease, along with 110 women and men from control, nonpolluted areas. The mean age was 70 years, and Cd-U values were 8–11 mg/g creatinine for exposed and 2.5 mg/g creatinine for nonexposed subjects. Results showed that indices of bone mineral content and density, obtained by microdensitometry, were significantly lower in cadmium-exposed men and women with renal dysfunction than in the nonexposed subjects. The second study involved 203 cadmium-exposed subjects with renal dysfunction and 80 nonexposed subjects. Multivariate analyses performed to evaluate relationships between parameters of renal dysfunction and multiple measures of bone mineral content and density obtained by microdensitometry showed a significant relationship between the extent of renal dysfunction and osteopenia.

The third study demonstrated that, as cadmium-induced renal damage increased (higher urinary β_2-microglobulin and lower %TRP), concentrations of 1,25$(OH)_2$-vitamin D in serum decreased, with a concomitant increase in parathyroid hormone. Results indicated that disturbances in vitamin D and parathyroid hormone may play a role in cadmium-induced bone damage. The extensive database presented essentially showed that, in both men and women exposed to cadmium to the point where they have cadmium-induced renal damage, the extent of their renal damage is directly related to the extent of their osteopenia, implying a causal role of their renal damage in the development of osteopenia.

A model for osteoporosis in dogs induced by cadmium and estrogen deficits was described. A database of studies in mice demonstrates that the female skeleton is sensitized to demineralizing effects of dietary cadmium both during periods of pregnancy and lactation and after removal of the ovaries (to simulate menopausal hormonal changes in humans). Studies with ^{45}Ca showed that the release of ^{45}Ca from bone occurred within 96 hr of the start of cadmium exposure and therefore preceded the renal damage characterized to occur after longer periods of exposure to dietary cadmium.

A study to evaluate combined effects of estrogen depletion (by ovariectomy) and cadmium exposure on bone of elderly female beagles (7–9 years old) whose skeletons were prelabeled with ^{45}Ca showed that serum ^{45}Ca levels were significantly increased within 96 hr of exposure to cadmium in drinking water (15 mg/l); the response was greatest in ovariectomized animals and was not mediated by changes in the calciotropic hormones [1,25$(OH)_2$-vitamin D, parathyroid hormone, calcitonin]. Mean blood cadmium concentrations were 3–8 mg/l during the earliest bone response and 13–15 mg/l 6 months later, providing a way to relate results to human exposures. Sequential *in vivo* dual photon absorptiometry measurements of tibia and lumbar vertebral bone mineral density (BMD) were made in each animal. The greatest decrease in BMD occurred in the ovariectomized animals exposed to cadmium for 7 months (-7% to -15%). Early histomorphometric analyses indicate that soon after cadmium exposure (4 weeks of drinking water Cd) and 3.5 months after ovariectomy, bone formation parameters were increased 1.5- to 4-fold by hormone depletion, independent of cadmium exposure. Histomorphometric analyses are continuing to ultimately demonstrate whether bone changes observed by *in vivo* BMD measurements and serum ^{45}Ca analyses correlate with changes in bone formation and resorption.

Aluminum

Osteopenic aluminum-related bone disorders, which include the renal osteodystrophic diseases low-turnover osteomalacia (LTOM) and aplastic bone disease occur in up to 20 and 33%, respectively, of the dialysis-dependent patients in North America. There are compelling data in support of a role of aluminum in the etiopathology of these diseases, including clinical data showing an association of aluminum exposure with disease incidence, an *in vivo* rat model for LTOM associated with aluminum exposure, and an *in vitro* system supporting an osteopenic response to aluminum. However, results of ambitious studies of straightforward design in beagle dogs gave contradicting results. In these experiments, osteomalacia was induced in dogs by giving them a diet deficient in both vitamin D and calcium. The dogs received aluminum by intravenous injection (3 mg/kg/week), resulting in deposition of aluminum on the bone-osteoid interface (shown histologically by bone biopsy), as occurs in renal dialysis patients. The dogs were then put on a replete diet to see if the aluminum on the bone surface would impede remineralization. Results clearly showed that bone healed with no problem directly over the layer of aluminum.

In a second study, dogs were injected with aluminum at 0.75 or 1.25 mg/kg/day three times per week for 16 weeks. At both doses, there was evidence of new bone formation at 8 weeks, with arborization of trabeculae and an increase in bone volume at the higher dose. This response did not occur in parathyroidectomized animals. When the dogs were taken off aluminum, the woven, osteoid-containing bone (formed in the presence of aluminum) mineralized. In addition, there was an increased proliferation of osteoblastlike MC3T3E1 cells in response to aluminum, in keeping with the *in vivo* findings.

Fluoride

In a detailed review of the epidemiological database for the role of fluoride in the pathogenesis of osteoporosis, two questions were evaluated: Is the current EPA maxi-

mum permitted level of fluoride in drinking water of 4 mg/l too high? Should fluoride be approved for treatment of osteoporosis? Although some studies have shown small increases in hip fracture rate with increasing fluoride intake from natural or adjusted sources of fluoride in drinking water, overall conclusions were that, at levels (1 mg/l) typically used in artificially fluoridated or many naturally fluoridated water supplies, there is weak or no evidence that fluoride induces more bone fractures than in nonfluoridated drinking water. It was also concluded that there is no basis to recommend that EPA lower the current 4 mg/l maximum containment level.

With respect to studies of fluoride for treatment of osteoporosis, the design of early studies was criticized because they were not randomized, controlled clinical trials. However, four recent randomized studies showed that fluoride treatment did not cause a statistically significant reduction in vertebral fractures. A recent study is being published by Pak et al. in which sodium fluoride administered in a slow release form significantly reduced vertebral fractures, indicating that slow-release fluoride may be a promising treatment for osteoporosis.

In the final two sessions of the conference, government activities on osteoporosis were reviewed. The National Institute of Arthritis and Metabolic and Skin Disease is supporting a study of osteoporotic fractures. This is the largest cohort study focused on osteoporosis and is currently collecting data on risk factors for fractures. Information collected to date has provided information about the etiology of falls and fractures, the sites in the body where osteoporotic fractures occur most commonly, and the role of bone density as a risk factor for fractures. The Women's Health initiative, the largest trans-National Institutes of Health study, will investigate osteoporosis, as well as cardiovascular disease and breast cancer in a large multicenter trial and observational study. Two of the interventions will have particular relevance to osteoporosis: hormone replacement and calcium/vitamin D supplementation. The study will investigate the significance and clinical applications of new genetic and biochemical markers of osteoporosis such as osteocalcin.

Conclusions

The potential link between lead exposure and osteoporosis is reasonable, based on our current understanding of osteoporosis and lead toxicity. Human and animal studies provide strong evidence for effects of lead on the endocrine regulation of bone mineral homeostasis, bone growth, and skeletal toxicity. These studies are in accord with current understanding of the cellular and molecular mechanisms of lead toxicity in bone and other cells.

Environmental levels of cadmium exposure in areas of previous contamination from smelter activities may affect calcium metabolism in the kidney, resulting in small increases in urinary calcium excretion. Epidemiology studies from Japan demonstrate that once renal tubular dysfunction appears in response to cadmium exposure, hypercalciuria and osteopenia follow. This response may be mediated by decreases in circulating levels of 1,25 $(OH)_2$-vitamin D, with concomitant increases in parathyroid hormone. Results of experimental studies support the conclusion that cadmium causes bone loss early after the start of dietary cadmium exposure (within 96 hr), before the start of cadmium-induced renal damage typified by increased urinary excretion of NAG, β_2-microglobulin, and amino acids. In addition, the bone demineralization response to cadmium is increased in females during pregnancy and lactation and in elderly females after menopausal hormone depletion, making females at greater risk of cadmium-induced bone loss than males.

The relationship between aluminum and bone disease, particularly osteoporosis, is currently not clear. The decrease in circulating parathyroid hormone concentrations that accompanies both low turnover osteomalacia and aplastic bone disease may be the result of an aluminum-mediated decrease in parathyroid hormone levels. However, the connection between aluminum exposure and bone disease should not be underestimated. There is seldom a patient with clear evidence of aluminum deposition in bone who does not also have bone disease.

Fluoride at levels found in drinking water does not appear to be associated with increases in bone fractures, and at intake levels about 10 times higher (50–80 mg/day), fluoride may decrease fractures when given in a slow release form.

Adequate dietary calcium is important early in life for bone formation, but calcium supplementation cannot repair other nutrient deficiencies or offset estrogen deprivation as occurs in the immediate postmenopausal period. Dietary sodium increases urinary calcium excretion.

Experimental studies suggest that there is direct estrogen-receptor-mediated action that influences bone density. Exposure to diethylstilbestrol and other environmental estrogens and estrogen agonists early in life may influence cell programming in estrogen target tissues including bone and change the risk for osteoporosis later in life.

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Future Research Directions

There are few experimental and clinical studies that are specifically designed to investigate the contribution of the environmental risk factors for osteoporosis identified at this conference. Research needs include the use of animal models to more clearly define the effects of these metals, particularly lead and cadmium, on the conservation of bone mineral (using tracer and histomorphometry) and to clarify dose–response relationships and exposure timing/duration factors most critical to the skeletal toxicity of these metals. There is a need to further investigate metal/environmental effects on bone cell function, including the endocrine, paracrine, and autocrine regulation, and to identify confounding and contributing factors that might exacerbate or minimize the development of osteoporosis (diet, genetics, pregnancy, lactation, etc.). There is a need to apply biomarkers for skeletal dysfunction in studies of effects of environmental contaminants on bone.

Because the etiology of osteoporosis is largely unknown and the risk factors are numerous, sophisticated epidemiological studies are also needed to establish the existence of a relationship between diseases and toxic metal exposures, particularly lead and cadmium. Studies should be conducted on metal exposure during childhood and adolescence that might alter peak bone mass and occupational exposure to metals culminating in high bone levels that might modulate bone loss.

Finally, it is important to consider the potential role of osteoporosis in the development of lead toxicity. Epidemiological studies suggest that lead is released from the skeleton to blood during bone loss. Thus, studies to determine the magnitude of skeletal lead release and the contribution of skeletal lead to lead toxicity of the aging nervous, renal, immune, and endocrine systems should be considered. Other research questions are whether the aging nervous system, like developing systems, is more susceptible to lead and whether exposure to lead and other toxic metals such as cadmium, and aluminum, and fluoride reduce the reserve capacity of organs and tissues and thereby exacerbate the aging process or the consequences of aging.

Although the beneficial effects of dietary calcium supplementation in young people are recognized, there is a need to learn more about the role of calcium supplementation above present dietary requirements on bone metabolism and its potential role in enhancing bone density later in life (post-menopause).

Studies on the mechanism of action of estrogens on receptors in bone should be continued, with emphasis on natural estrogens and estrogen agonists occurring in the environment. The time course of any effects have to be evaluated as well.

Finally, studies of other etiologic factors in osteoporosis should include relationships to environmental factors that presumably can be controlled.